

## ABSTRACT

The development and standardization of antibody interferent testing provides an invaluable platform for the provision of consistent, high quality antibody assays and reagents for use in current biodetection platforms, as well as in the development and validation of future systems. This study examined how certain environmental contaminants affect the binding affinity of specific SEB antibodies to their antigens and illustrates how the issue of contaminants remain a critical aspect to consider when developing fieldable antibody-based assays for the detection of biothreats.

## BACKGROUND

While antibody capture has been regarded as the “gold Standard” in detection of biological threats agents, very little is known about the effects that common environmental contaminants may have on their performance. Here, we report the results of using high throughput BioLayer Interferometry (BLI) technology to assess the effect of six common environmental substances on the binding of eight Staphylococcal enterotoxin B (SEB) antibodies to their target SEB antigen. Antibodies incorporated into fielded biological threat detection assays are often exposed to wide variety of contaminating interferents that may impair an assay’s target sensitivity. Because of this, it is critical that antibody-based assays be validated to be both accurate and reliable, regardless of environmental factors. To date, the selection of antibodies for inclusion in an assay format has primarily relied on an antibody’s performance in an enzyme-linked immunosorbent assay (ELISA) with little regard for quantifying the full spectrum of variables affecting antibody-antigen interaction. Here, we report the results of using high throughput BioLayer Interferometry (BLI) technology to assess the effect of twenty six common environmental substances on the binding of eight Staphylococcal enterotoxin B (SEB) antibodies to their target SEB antigen.



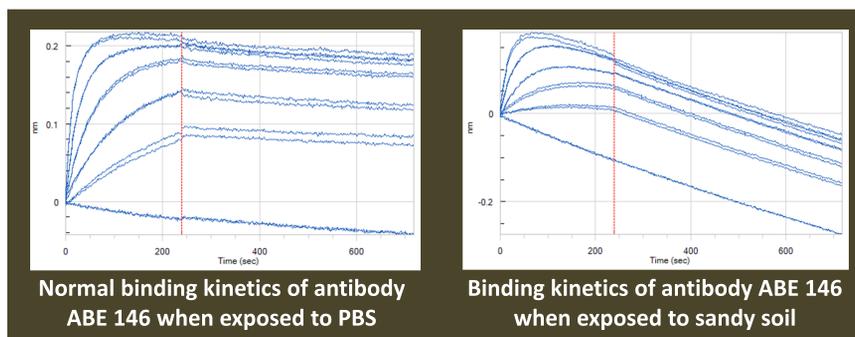
### SEB poses a significant biological threat.

- ❖ Environmentally resilient: survives temperatures up to 100°C
- ❖ Highly transmissible: Food, Water, and Air born
- ❖ Very Low ID50: Inhalation ID50 nanogram or lower

SEB, an enterotoxin produced primarily by the gram positive bacteria *Staphylococcus aureus*, triggers an excessive cellular immune response potentially leading to toxic shock syndrome. The excessive immune response that ensues is believed to cause the toxic effects of SEB. Because of the low dose of SEB needed to incapacitate people, SEB has been considered a potential biological threat agent. Given *Staphylococcus aureus*’ environmental route of transmission through food and water, as well as its ability to be aerosolized, the development of assays for detecting/diagnosing SEA/SEB in various sampling matrices has been on ongoing challenge. In this study, eight Staphylococcal enterotoxin B (SEB) antibodies were tested against the Critical Reagents Program’s (CRP) panel of twenty-six interferents using high throughput BioLayer Interferometry (BLI) technology to test the kinetics of specific SEB antibodies in complex matrices.

## MATERIALS AND METHODS

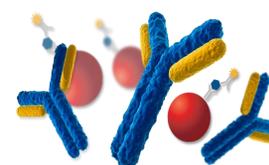
- Biosensors were pre-soaked in a 1:20 dilution of interferent in 10X Kinetics Buffer (interferent buffer) for at least 20 minutes before each run.
- Each antibody was directly immobilized onto its respective sensor, by dipping the tips into the antibodies for a total of 120 seconds.
- After quenching with excess biotin to eliminate non-specific binding. A baseline was then established for 60 seconds in interferent buffer.
- The sensors then dip into the SEB wells for 240 seconds of association.
- There were six concentrations of SEB in interferent buffer using a two-fold dilution series beginning with 1.12 µg/mL and ending with 0 µg/mL.
- Dissociation was carried out for 480 seconds in the same interferent buffer wells used for the baseline



- Use BLI to assess the kinetics of antibodies in complex matrices
- Eliminate refractive index problems
- Eliminate microfluidics
- Cost efficient sensors

The BLI instrument measures the rate of binding or association of the analyte in solution to the immobilized ligand ( $k_a$ ) and the rate that the complex dissociates ( $k_d$ ). These measurements are used to determine the kinetic equilibrium constant of the interaction ( $K_D$ ).

Specimen No.	Catalog Number	Material
ABE 091	AB-SEB-MAB	Anti-SEB
ABE 146	AB-AR-SEB	Anti-SEB
ABE 152	AB-AR-SEB	Anti-SEB
ABE 166	AB-AR-SEB	Anti-SEB
ABE 190	AB-AR-SEB	Anti-SEB
ABE 252	AB-AR-SEB	Anti-SEB
ABE 270	AB-AR-SEB	Anti-SEB
ABE 280	AB-AR-SEB	Anti-SEB



### Kinetic Constant Statistical Analysis

To determine whether or not the difference in  $K_D$  between interferents for an antibody was statistically significant from a normal binding reaction, each sensorgram was analyzed individually to generate a  $K_D$  for each separate concentration of analyte. A Generalized ESD Test for outliers was then performed to remove any extraneous data and a standard t-test was used to determine significance based on the baseline  $K_D$  value of the antibody in PBS. The following table displays the findings with red highlighted areas representing a statistically significant difference in  $K_D$  value from the baseline.

## RESULTS

PBS, pH 7.4	ABE146		ABE152		ABE166		ABE190		ABE252		ABE270		ABE280		ABE91	
	KD	P-value														
Aspergillus niger	1.96E-10	0.74	3.14E-10	0.27	5.17E-10	0.69	9.38E-10	0.77	2.51E-10	0.14	5.92E-10	0.78	3.84E-10	0.16	2.92E-09	0.22
BHI Broth	4.39E-10	0.13	5.95E-10	0.46	8.4E-10	0.13	7.76E-10	0.91	5.86E-10	0.29	6.72E-10	0.16	1.36E-09	0.31	7E-09	0.39
Brucella Broth	2.61E-10	0.36	3.28E-10	0.22	1.18E-09	0.32	8.31E-10	0.87	3.72E-10	0.14	6.03E-10	0.58	4.99E-10	0.21	9.97E-09	0.55
BSA, Fraction V	2.91E-10	0.19	4.59E-10	0.36	5.74E-10	0.19	9.96E-10	0.61	5.1E-10	0.26	5.4E-10	0.50	1.48E-09	0.54	8.46E-10	0.12
Burning Diesel	1.88E-10	0.55	6.18E-10	0.46	9.68E-10	0.34	3.68E-10	0.22	7.14E-10	0.30	1.56E-09	0.43	4.5E-10	0.16	4.91E-09	0.30
Burning Fog Oil	5.69E-10	0.04	3.38E-10	0.28	6.7E-10	0.30	4.53E-10	0.41	6.17E-10	0.24	--	0.00	1.65E-09	0.45	--	0.00
Burning Rags	2.91E-10	0.16	2.66E-10	0.19	8.97E-10	0.35	6.6E-10	0.90	3.81E-10	0.15	8.11E-10	0.41	5E-10	0.22	7.89E-09	0.43
Burning Rubber	2.09E-10	0.88	1.65E-09	0.66	5.63E-10	0.26	4.9E-10	0.45	1.86E-09	0.88	5.35E-10	0.57	8.93E-10	0.95	4.82E-09	0.29
Burning Veg	2.6E-10	0.39	2.14E-10	0.17	2.8E-10	0.11	2.99E-10	0.14	5.1E-10	0.19	9.99E-10	0.35	4.77E-10	0.21	2.39E-09	0.16
Clay Soil	--	0.00	--	0.00	--	0.00	--	0.00	--	0.00	--	0.00	--	0.00	--	0.00
G media w/ trace minerals	1.45E-10	0.13	4.09E-10	0.30	8.79E-10	0.29	8.42E-10	0.80	1.12E-09	0.63	5.98E-10	0.31	7.26E-10	0.63	4.01E-09	0.20
Green Signal Smoke	2.32E-10	0.74	6.64E-10	0.50	4.07E-10	0.93	5.34E-10	0.53	8.7E-10	0.43	5.77E-10	0.22	9.4E-10	0.95	1.26E-08	0.74
HC Smoke	--	0.00	--	0.00	--	0.00	--	0.00	--	0.00	3.04E-09	0.00	--	0.00	2.6E-09	0.28
Loamy soil	--	0.00	--	0.00	--	0.00	--	0.00	--	0.00	--	0.00	--	0.00	4.36E-09	0.23
Malathion	2.82E-10	0.19	7.51E-10	0.60	5.55E-10	0.32	5.1E-10	0.50	1.95E-09	0.85	4.53E-10	0.65	1.21E-09	0.56	9.54E-09	0.50
Nutrient Broth	3.43E-10	0.06	5.38E-10	0.37	4.76E-10	0.38	3.83E-10	0.24	7.36E-10	0.30	9.27E-10	0.42	6.37E-10	0.42	7.22E-10	0.12
Red Signal Smoke	2.05E-10	0.89	6.17E-10	0.49	2.92E-10	0.24	1.02E-09	0.56	7.69E-10	0.37	5.05E-10	0.79	5.1E-10	0.27	1.47E-08	0.85
Sage Pollen	2.97E-10	0.20	6.62E-10	0.53	4.6E-10	0.57	4.18E-10	0.32	7.93E-10	0.35	--	0.00	6.66E-10	0.50	--	0.00
Sandy Soil	5.68E-09	0.00	5.62E-09	0.00	4.79E-09	0.00	4.7E-09	0.00	3.64E-09	0.00	6.12E-09	0.00	--	0.00	3.73E-09	0.25
Tryptic Soy Broth	1.84E-10	0.49	7.64E-10	0.60	6.37E-10	0.25	4.01E-10	0.26	6.35E-10	0.24	5.38E-10	0.44	8.62E-10	0.88	5.2E-09	0.30
Tween 80, 1% in PBS	9.03E-11	0.01	3.85E-10	0.26	3.56E-10	0.74	2.72E-10	0.12	1.06E-09	0.57	5.87E-10	0.69	5.04E-10	0.21	6.39E-09	0.36
Vero Cell Supernatant	4.87E-10	0.00	1.32E-09	0.87	7.33E-10	0.05	8.27E-10	0.79	1.09E-09	0.59	6.48E-10	0.02	2.1E-09	0.18	8.57E-09	0.44
Violet Signal Smoke	2.17E-10	0.97	3.5E-10	0.26	5.91E-10	0.19	4.1E-10	0.30	9.15E-10	0.42	4.13E-11	0.00	1.66E-09	0.39	2.91E-10	0.11
Water	2.2E-10	0.92	2.38E-10	0.18	3.28E-10	0.43	1.15E-09	0.68	4.22E-10	0.16	6.51E-10	0.48	4.07E-10	0.13	5.74E-09	0.28
Yellow Signal Smoke	3.1E-10	0.00	3.26E-10	0.35	3.52E-10	0.64	3.82E-10	0.30	3.89E-10	0.18	4.25E-11	0.00	1.19E-09	0.61	2.61E-11	0.10

## CONCLUSION

- ❑ BLI was used to test 8 CRP SEB antibodies against the CRP panel of 26 interferents.
- ❑ The most significant effects on antibody-antigen binding were observed on exposure to HC gas, clay, sandy soil, and loamy soil.
- ❑ The four major interferents appeared to generally effect all of the SEB antibody-antigen binding results, regardless of the SEB antibody, while other interferents showed selective effects on some SEB antibodies, but not others.



## ACKNOWLEDGEMENTS

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